Zoosyst. Evol. 101 (2) 2025, 583–596 | DOI 10.3897/zse.101.144271

PENSOFT

MUSEUM FÜR NATURKUNDE BERLIN

Vastnema crassicutaneum gen. et sp. nov. (Nematoda, Dorylaimida, Dorylaimidae), an interesting new taxon from natural areas of Vietnam

Tam T. T. Vu[1,2], Joaquín Abolafia[3], Anh D. Nguyen[1,2], Thi Mai Linh Le[1,2], Reyes Peña-Santiago[4]

1 *Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
2 *Graduate University of Science and Technology, Vietnam Academy of Science and Technology. 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
3 *Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n, Edificio B3, 23071- Jaén, Spain*
4 *Universidad de Jaén, Jaén, Spain*

https://zoobank.org/B9E64763-486C-429E-AC47-F78ED360333F

Corresponding author: Tam T. T. Vu (vtam7572@gmail.com)

Academic editor: A. Schmidt-Rhaesa ♦ **Received** 12 December 2024 ♦ **Accepted** 24 January 2025 ♦ **Published** 28 February 2025

Abstract

A new dorylaimid taxon, *Vastnema crassicutaneum* gen. et sp. nov., collected in natural habitats of Vietnam, is characterized, including morphological description, morphometrics, SEM observations, and molecular (18S-, 28S-rDNA) analyses. *Vastnema* gen. nov. is distinguished and separated from its relatives by a combination of key traits: large size (body 4.79–6.35 mm long), lip region tapering and continuous with the adjoining body, very short neck (*b*-ratio 6.3–8.7), presence of a peculiar uterine differentiation, pre-equatorial ($V = 38–44$) vulva, and 45–58 stomata-like ventromedian supplements. The new species is characterized by its three-layered cuticle, 12.5–15.5 µm thick at the anterior region; lip region 26–31 µm wide; odontostyle 49–60 µm long, or 1.8–2.0 times the lip region diameter; neck 627–888 µm long; pharyngeal expansion occupying 46–49% of the total neck length; uterus complex and 571–804 µm, or 3.2–4.3 body diameters long; longitudinal vulva; tail short and rounded in both sexes (39–56 µm, $c = 98–140$, $c' = 0.5–0.7$); and spicules 138–160 µm long. Both morphological and molecular data support a close relationship of the new taxon with some Labronematinae representatives but also reveal that internal and external relationships of Dorylaimidae might be more complex than traditionally assumed.

Key Words

Labronematinae, LSU, morphometrics, phylogeny, SEM, SSU, taxonomy

Introduction

Dorylaims, the members of the order Dorylaimida, are probably the most diverse nematode taxon, being present everywhere on the six continents and in all kinds of continental habitats, either freshwater sediments and/or arable and pristine soils (Peña-Santiago 2021). Traditionally, the study of dorylaimid fauna of Vietnam did not receive too much attention. Actually, Nguyen et al. (2014) compiled previous records of only 25 species and 15 genera. Nevertheless, the inventory of species significantly increased throughout the last decade by, among other contributions, Álvarez-Ortega et al. (2015, 2016), Nguyen et al. (2016, 2017), Nguyen and Peña-Santiago (2018, 2020), Hoang et al. (2019), and Vu et al. (2024).

Several populations of an interesting dorylaimid form were collected in the course of a nematological survey conducted to explore the nematode fauna of Vietnam. Its study revealed that it was distinguished by a unique combination of features and that it was not comparable to any known genus. Thus, this contribution aims to characterize the new taxon and to discuss its evolutionary relationships. The results are presented in the following.

Copyright *Vu, T.T.T. et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Materials and methods

Sampling, extraction, and processing of nematodes

A total of 26 adult specimens, collected in soil samples from three natural areas of Vietnam, were studied. Nematodes were extracted using a modified Baermann funnel technique, heat killed, and fixed in TAF solution (Southey 1986) for morphological observations or in a DESS mixture (Yoder et al. 2006) for molecular analyses. Then, specimens were transferred to anhydrous glycerol (Seinhorst 1959, 1962), mounted on glass slides for their observation with light microscopy, and measured and photographed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast optics, a drawing tube (camera lucida), and a DS digital camera. Line drawings were made from taken photomicrographs and/or sketches obtained by means of camera lucida. Morphometrics include Demanian indices and other measurements and ratios, some of them presented in a separate table, while others form part of the literal description of species. All measurements were recorded in µm, except body length in mm. After filming and taking pictures, selected specimens were submitted to molecular studies.

DNA extraction, polymerase chain reaction (PCR), and sequencing

Nematode DNA was extracted from a single individual as described by Holterman et al. (2006), and DNA extracts were stored at –20 °C until used as PCR templates. The D2, D3 expansion segment of 28S rDNA and 18S was amplified using the forward D2A (5′–ACAAGTACCGTGGGGAAAGTTG–3′) and reverse D3B (5′–TCGG AAGGAACCAGCTACTA–3′) primers (Subbotin et al. 2006), and the 18S rDNA fragment was amplified using the primers 18S (18F: 5′-TCTAGAGCTAATACATGCAC-3′/18R: 5′-TACGGAAACCTTGTTACGAC-3′) (Floyd et al. 2005). All PCR reactions contained 12.5 µl of Hot-Start Green PCR Master Mix (2x) (Promega, USA), 1 µl of the forward and reverse primer (10 µM each), the 3 µl DNA template, and sterile Milli-Q water to 25 µl of the total volume. All PCR reactions were performed in a SimpliAmp thermal cycler (Thermo Fisher Scientific) as follows: an initial denaturation step at 95 °C for 4 min, followed by 40 cycles at 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 60 s with a final incubation for 5 min at 72 °C. Amplicons were visualized under UV illumination after simple safe gel staining and gel electrophoresis. Purified PCR products were sent to Apical Scientific Company for sequencing (Selangor, Malaysia). After sequencing, the obtained rDNA sequence fragments were deposited in GenBank.

Phylogenetic analyses

For exploring phylogenetic relationships, analyses were based on 18S and 28S rDNA. The newly obtained sequences were manually edited using Chromas 2.6.6 (Technelysium, Queensland, 110 Australia) and aligned with other sequences available in GenBank using the ClustalW alignment tool implemented in MEGA11 (Kumar et al. 2021). Poorly aligned regions at extremes were removed from the alignments using MEGA11. The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba et al. 2012). The phylogenetic tree was generated with the Bayesian inference method using MrBayes 3.2.6 (Ronquist et al. 2012). The analysis under the generalized time reversible and invariant sites and gamma distribution (GTR + I + G) model was initiated with a random starting tree and run with the Markov chain Monte Carlo (Larget and Simon 1999) for 1×10^6 generations. The tree was visualized and saved with FigTree 1.4.4 (Rambaut 2018).

Results and discussion

Taxonomy and systematics

Family: Dorylaimidae de Man, 1876
Subfamily: Labronematinae Peña-Santiago & Álvarez-Ortega, 2014

***Vastnema* Vu, Abolafia & Peña-Santiago, gen. nov.**
https://zoobank.org/D80ECAAF-2697-4B75-9521-5EAAD6CB72CC

Type and only species. *Vastnema crassicutaneum* sp. nov.

Etymology. The genus name derives from VAST (Vietnam Academy of Science and Technology) and "*nema*".

Diagnosis. Dorylaimidae. Labronematinae. Large to very large-sized nematodes. Cuticle dorylaimid, very thick, three-layered. Lip region tapering, continuous with the adjoining body, with totally fused lips and very small cephalic papillae. Amphid aperture less than one-half of lip region diameter. Odontostyle strong, up to twice the lip region diameter, with wide aperture. Guiding ring, delicate, double. Odontophore rod-like, simple. Pharynx unusually short, entirely muscular, very gradually enlarging, with basal expansion occupying less than half of the total neck length. Female genital system di-ovarian with a peculiar uterine differentiation, well-developed *pars refringens vaginae*, and pre-equatorial longitudinal vulva. Tail similar in both sexes, short and rounded. Spicules dorylaimid, slender. Ventromedian supplements 45–58 in number, very shortly spaced, small, not typically mammiform, with hiatus.

Morphological separation from its closest genera

The general appearance of the new genus (large size, strong odontostyle, double guiding ring, rounded tail in both sexes) resembles the morphological pattern observed in some members of Labronematinae Peña-Santiago & Álvarez-Ortega, 2014, especially *Labronema* Thorne, 1939, and *Nevadanema* Álvarez-Ortega & Peña-Santiago, 2012. It differs from *Labronema*, a very heterogeneous genus (needing a good revision, *cf.* Andrássy 2009, 2011) in its larger size (body 4.79–6.35 mm *vs.* very occasionally exceeding 4.5 mm long), lip region tapering, with rounded anterior margin and continuous with the adjoining body (*vs.* not tapering, with truncate or even somewhat sunken anterior margin and often offset by deep constriction), very short neck (b = 6.3–8.7 *vs.* b-ratio always less than 6.3 and very sporadically more than 5.0), presence (*vs.* absence) of a peculiar uterine differentiation, pre-equatorial (V = 38–44 *vs.* equatorial or post-equatorial vulva, V-ratio never under 44 and very often *ca* 50 or more), and much more ventromedian supplements (45–58 *vs.* a maximum of 31) with different shape (very low and resembling stomata of tree leaves *vs.* typically mammiform). [See also the detailed separation of type species from some atypical members of *Labronema*.] The new genus is easily distinguishable from *Nevadanema* by its much larger general size (*vs.* body length 2.36–3.40 mm), lip region shape (*vs.* cap-like and offset by constriction), larger odontostyle aperture (more than one-third *vs.* less than one-fifth of total length), comparatively much shorter neck (*vs.* b = 3.6–4.5), pre-equatorial vulva (*vs.* V = 49–56), complex (*vs.* simple) uterus, and male as frequent as female (*vs.* male absent).

Descriptions of species

Vastnema crassicutaneum sp. nov.

https://zoobank.org/2BDC6FBA-31AA-4079-A389-CE3CC740BFB9

Figs 1–6

Material examined. Sixteen females and eight males from three locations, in good state of preservation, some specimens probably slightly flattened due to their large body, including wide diameter at mid-body.

Morphometrics. See Table 1.

Type population. (Nature Reserve Nam Xuan Lac, Cho Don district, Bac Can province) 11 females and seven males.

Holotype. Female adult, Northern Vietnam, Bac Can province, Cho Don district, Nam Xuan Lac nature reserve, December 2021 (coordinates 22°17'30"N, 105°31'06"E, elevation 800 m), soil from a wild forest.

Paratypes. Eleven females and seven males with the same collection details as the holotype.

Other material. All in Northern Vietnam. i) Ha Giang province, Bac Me district, Du Gia nature reserve, October 2023 (coordinates 22°43'13"N, 105°11'32"E, elevation 300 m); ii) Ninh Binh province, Cuc Phuong National Park, August 2019 (coordinates 20°21'06"N, 105°35'22"E, elevation 430 m), soil from a wild forest.

Description. Adult. Moderately slender to slender (a = 25–34) nematodes of large to very large size, 4.79–6.35 mm long. Body cylindrical, clearly narrowing towards the anterior end, much less towards the posterior extremity, as the tail is short and rounded. Upon fixation, habitus almost straight. Cuticle smooth (but a very fine transverse striation is appreciable with SEM), three-layered, consisting of thin outer and inner layers and much thicker intermediate layer, especially conspicuous at level of anterior region and tail, 12.5–15.5 µm thick at anterior region, 9–15 µm in midbody, and 14–20 µm on tail. Lateral chord 12–29 µm wide, occupying up to one-sixth (8–16%) of midbody diameter, lacking any differentiation. Body pores very small, the lateral ones arranged in a single row throughout the body length, a few dorsal and ventral pores are present at cervical region. Lip region rounded, occasionally slightly asymmetrical, visibly tapering, less than one-fourth (18–24%) of body diameter at neck base, 2.2–2.5 times as wide as high, continuous with the adjoining body or hardly offset by a very shallow depression. SEM observations: lips totally amalgamated, oral field wide as inner and outer labial papillae are quite close, perioral area not differentiated, labial papillae button-like, cephalic papillae appearing as very small pores (Fig. 6A–C). Amphidial fovea cup-like, its aperture 10.5–13.5 µm wide, occupying less than one-half (33–48%) of lip region diameter, appearing as a wide transverse slit visibly occluded at its middle. Cheilostom almost cylindrical, 32–40 µm long, with moderately thick walls, but lacking any other specialization. Odontostyle strong, 1.7–2.0 times longer than lip region diameter, 8.3–11.8 times as long as wide, 0.89–1.13% of body length, its aperture 19–27 µm long or less than one-half (36–48%) of the total length. Guiding ring, double, but very delicate and difficult to visualize in some specimens. Odontophore simple, rod-like, 1.3–1.6 times the odontostyle. A mucro 2.5–8.5 × 1.5–3 µm is present in several specimens at level of odontophore base (Fig. 2A, B, K). Pharynx comparatively very short (b = 6.3–8.7), strongly muscular, very gradually enlarging into the basal expansion that is 3.4–6.1 times as long as wide, 1.9–3.0 times longer than body diameter at neck base, and occupies up to one-half (46–49%) of the total neck length; pharyngeal gland nuclei located as follows: DO = 52–56, DN = 53–58, S_1N_1 = 67–69, S_1N_2 = 74–79, S_2N = 83–88. Nerve ring located at 228–308 µm or 33–36% of the total neck length from the anterior end. Pharyngo-intestinal junction consisting of a conical to somewhat cylindroid cardia 41–84 × 17–32 µm almost entirely enveloped by intestinal tissue, and a weak, ring-like structure around the junction of cardia to pharyngeal base.

Female. Genital system diovarian, with both branches equally and well-developed, the anterior 823–1250 µm long, or 16–24% of body length, the posterior 835–1153 µm long, or 15–21% of body length. Ovaries variable in length, 221–704 µm the anterior, 185–619 µm the posterior, often reaching and surpassing the sphincter level, with oocytes arranged first in several rows and then in a single one. Oviduct variably long too, 157–349 µm or 0.8–2.0 body diameters,

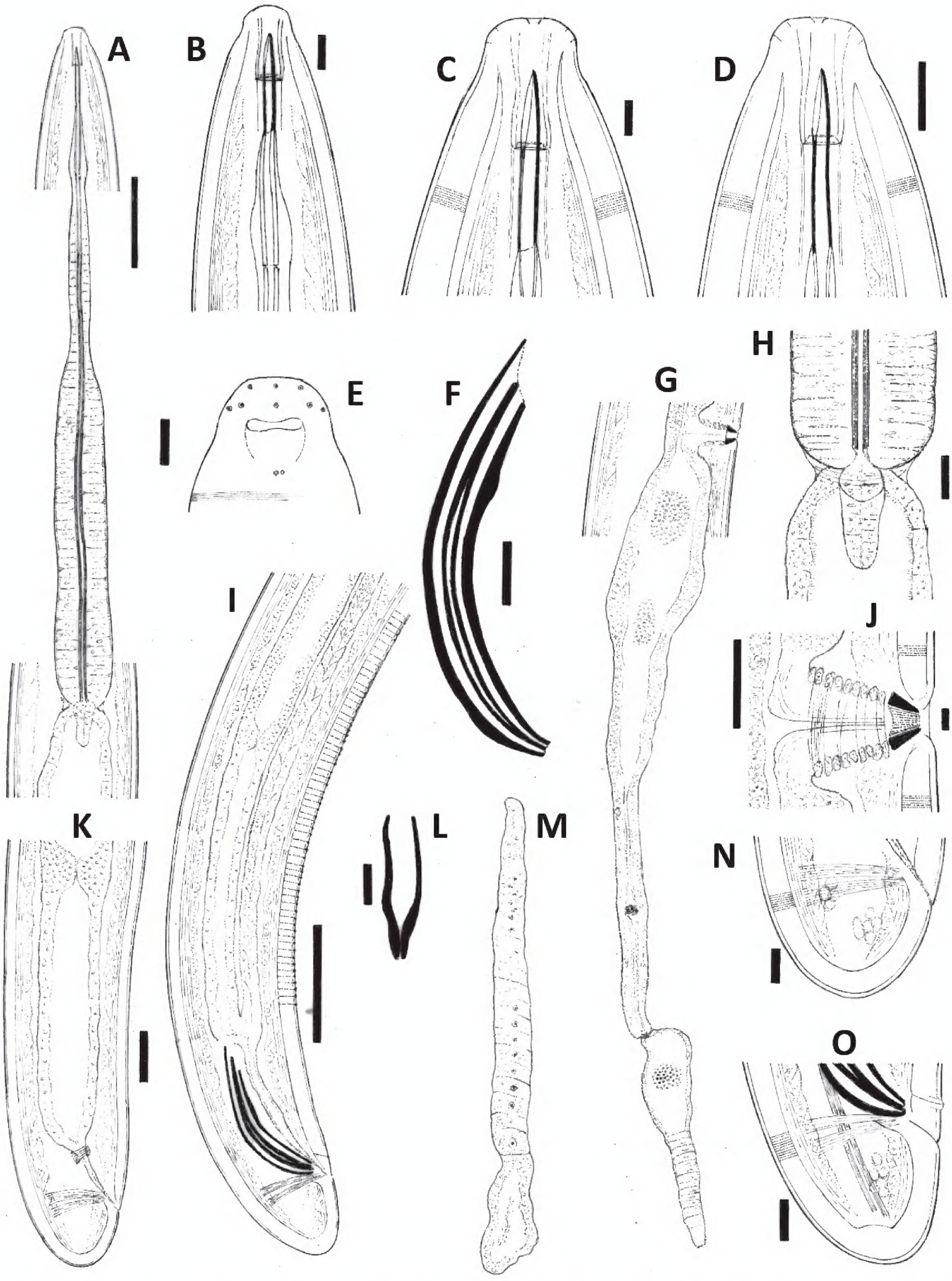

Figure 1. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam. **A.** Neck region; **B–D.** Anterior region, lateral median view; **E.** Anterior region, lateral surface view; **F.** Spicule; **G.** Female, posterior genital branch; **H.** Pharyngo-intestinal junction; **I.** Male, posterior body region; **J.** Vagina; **K.** Female, posterior body region; **L.** Lateral guiding piece; **M.** Posterior ovary; **N.** Female, caudal region; **O.** Male, caudal region. Scale bars: 100 µm (**A, G, I, M**); 20 µm (**B, D, F, H, N, O**); 10 µm (**C, E, J, L**); 50 µm (**K**).

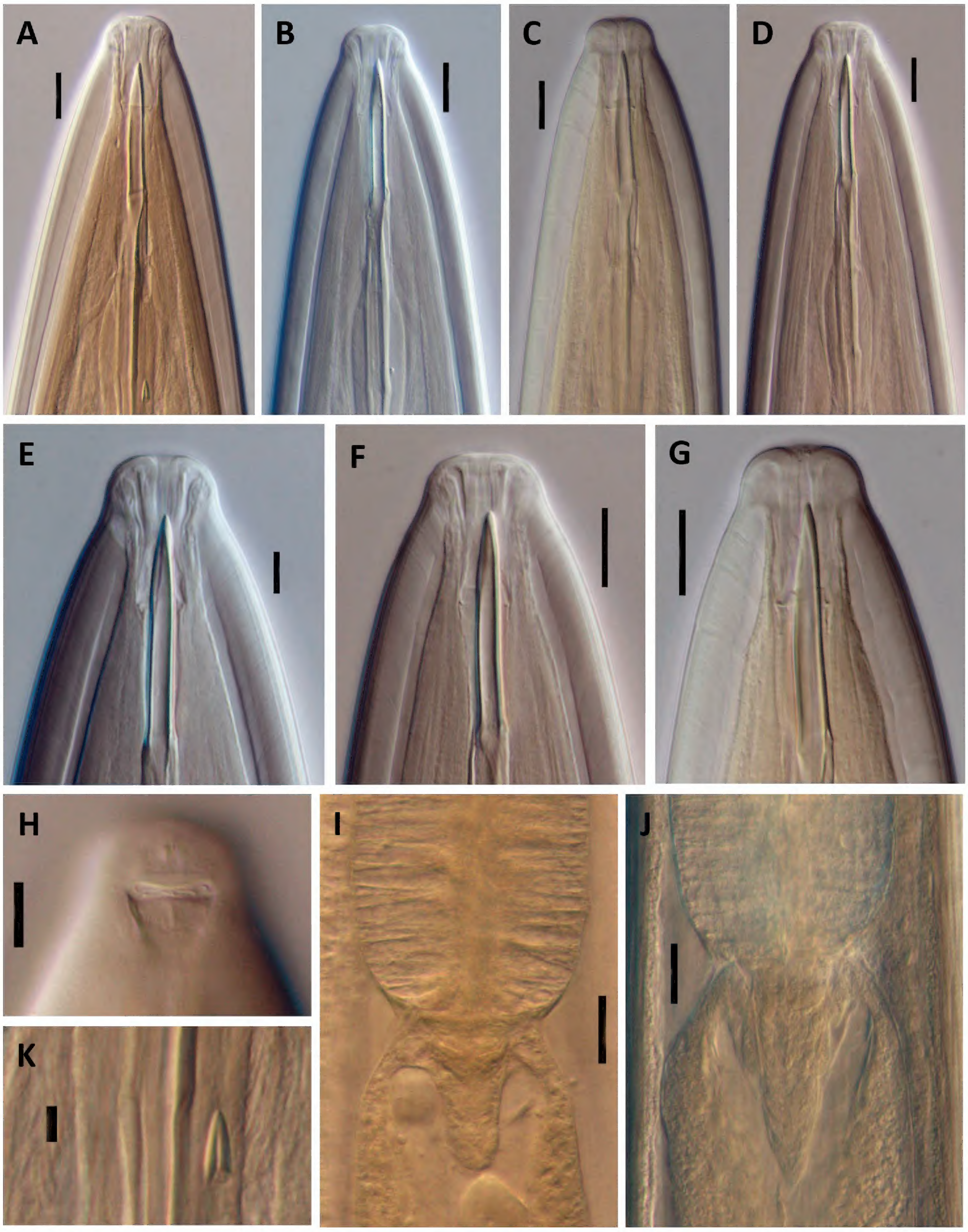

Figure 2. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam (LM). **A–G.** Anterior region, lateral median view; **H.** Anterior region, lateral surface view; **I, J.** Pharyngo-intestinal junction; **K.** Mucro located at odontophore base. Scale bars: 20 µm (**A–D, F, G, I, J**); 10 µm (**E, H**); 5 µm (**K**).

consisting of two sections almost equal in length: distal part made of prismatic cells and proximal one an elongated ***pars dilatata*** always with wide lumen inside and containing abundant sperm cells, therefore functioning as spermatheca. A weak sphincter separates the oviduct from the uterus. Uterus 571–804 µm or 3.2–4.3 body diameters long, bipartite, consisting of distal, longer, and slender region with narrow lumen and a proximal, shorter, and wider region with

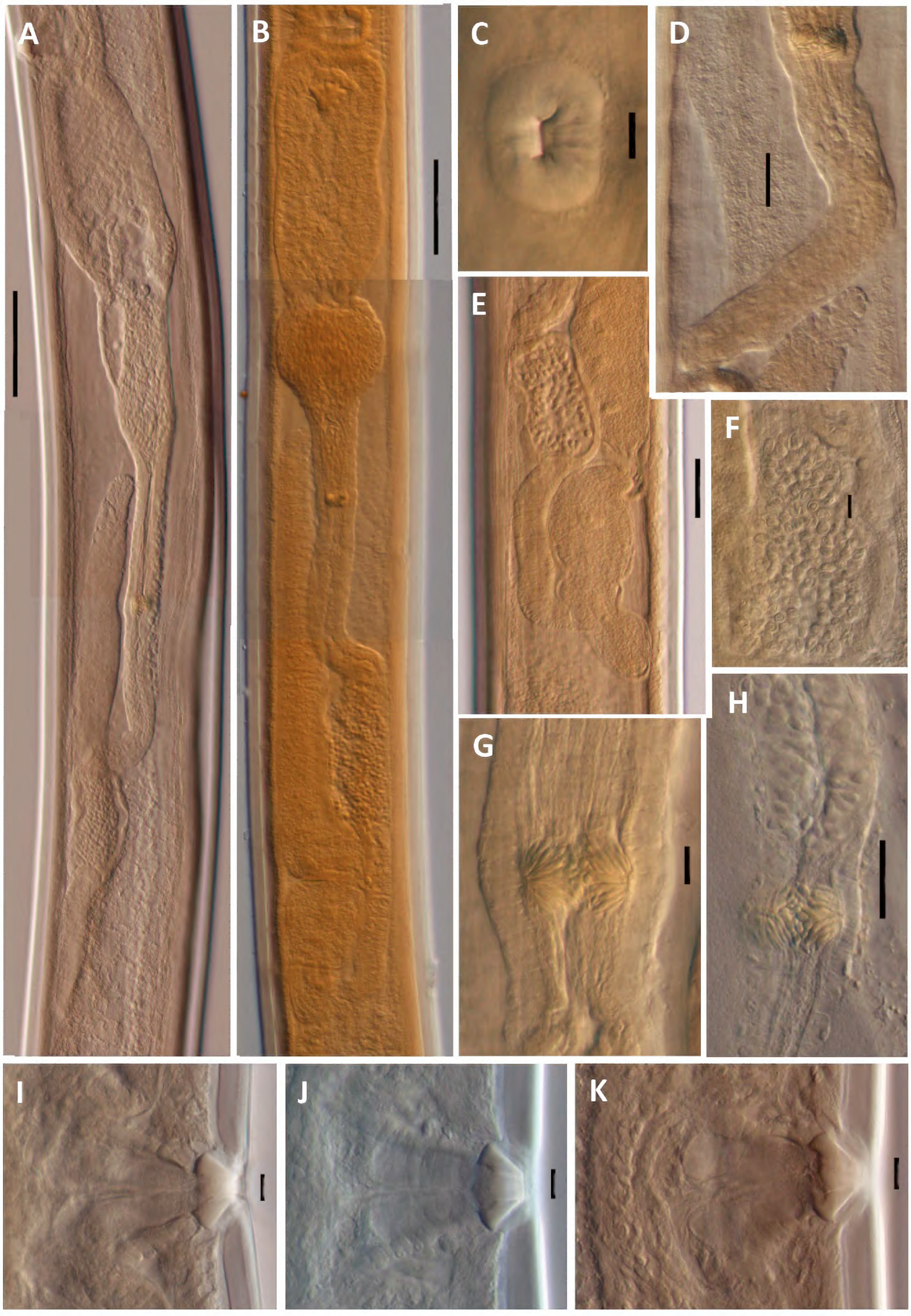

Figure 3. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam (LM, female genital system); **A, B.** Posterior branch; **C.** Vulva, ventral view; **D.** Oviduct-uterus junction; **E.** Oviduct; **F.** Sperm cells inside *pars dilatata oviductus*; **G, H.** Uterus differentiation; **I–K.** Vagina, lateral view. Scale bars: 100 µm (**A**); 10 µm (**C, F, G, I–K**); 20 µm (**D, H**); 50 µm (**E**).

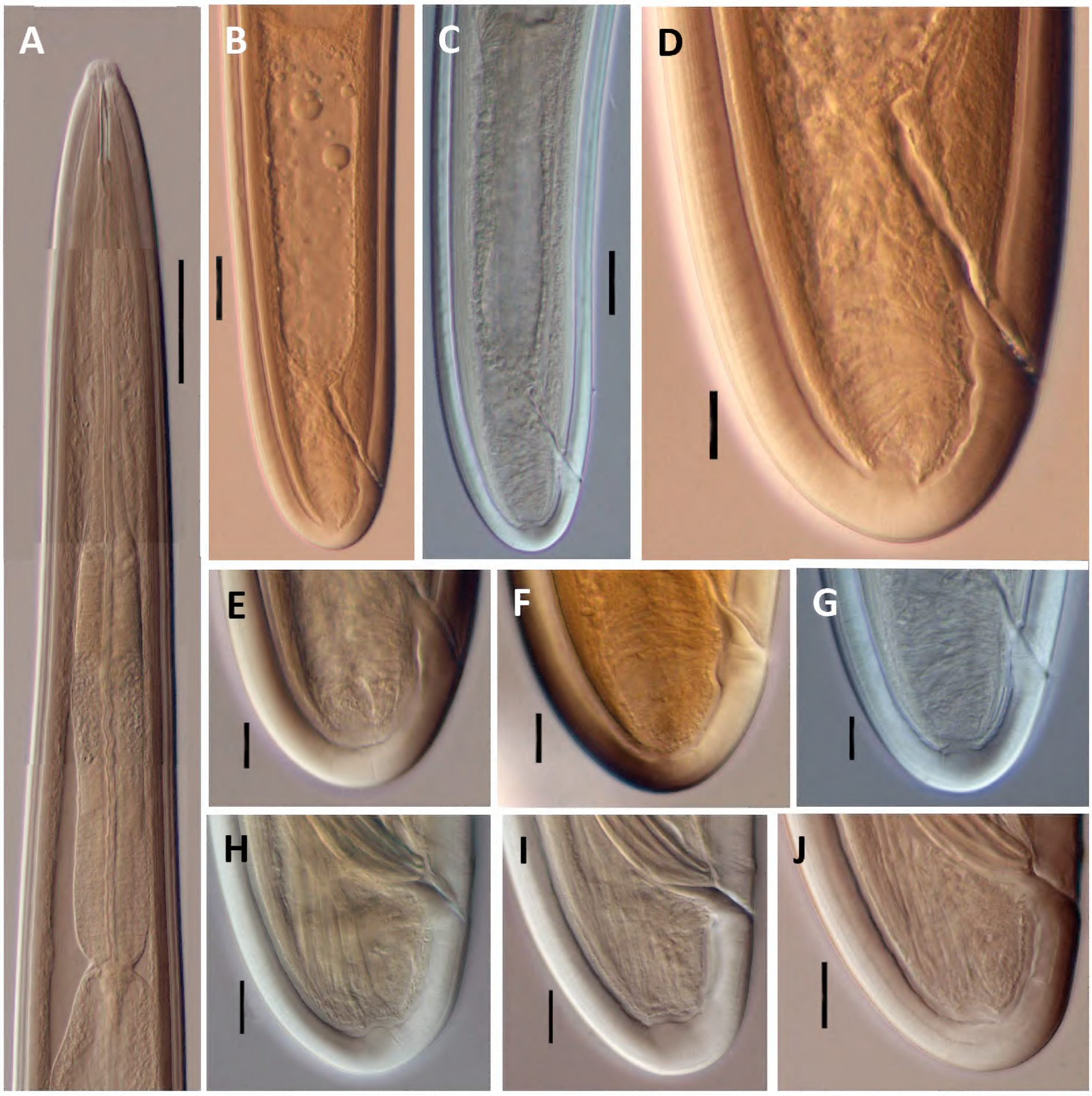

Figure 4. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam (LM, neck and posterior region); **A.** Neck region; **B, C.** Female, posterior body region; **D.** Female, rectum and caudal region; **E–G.** Female, caudal region; **H–J.** Male, caudal region. Scale bars: 100 µm (**A**); 50 µm (**B, C**); 20 µm (**D–J**).

broad lumen and often containing abundant sperm cells inside; distal region always bearing a very special differentiation, resembling the Z-like structure of some dorylaims, with a concentration of many small spindle-shaped elements visibly refringent that adopt a peculiar arrangement (Fig. 3A, B, G, H), all together measuring 13–18 × 22–26 µm. Vagina 54–73 µm long, to *ca* one-third (31–36%) of body diameter: ***pars proximalis*** 39–51 × 26–37 µm, with divergent walls surrounded by moderately developed circular musculature; ***pars refringens***, consisting of (lateral view) two separated triangular pieces 12–16 × 6–10 µm and with a combined width of 26–32 µm; ***pars distalis*** 5–10 µm long. Vulva longitudinal, slightly elliptical, 8–11 µm long. Prerectum 2.2–3.3, rectum 0.8–1.3 body diameters long. Anus a weakly arched transverse slit *ca* 15 µm long. Tail short and rounded, inner core 17–22 µm long, occupying less than half (37–48%) of tail length, caudal pores two pairs, at the middle of tail, one sublateral, another subdorsal.

Male. Prerectum 3.4–5.5, cloaca 1.3–1.5 times longer than body diameter at level of the cloacal aperture. Genital system diorchic with opposite testes. Sperm cells elliptical, 8 × 4.5 µm. In addition to the pre-cloacal pair, situated at 16–24 µm from the cloacal aperture, there is a series of 47–58, shortly spaced (7–14, exceptionally reaching 19 µm in between), very low, ventromedian supplements, the most posterior of them located at 101–154 µm from the adcloacal pair, thus with a perceptible hiatus. As seen with SEM, the ventromedian supplements are not mammiform

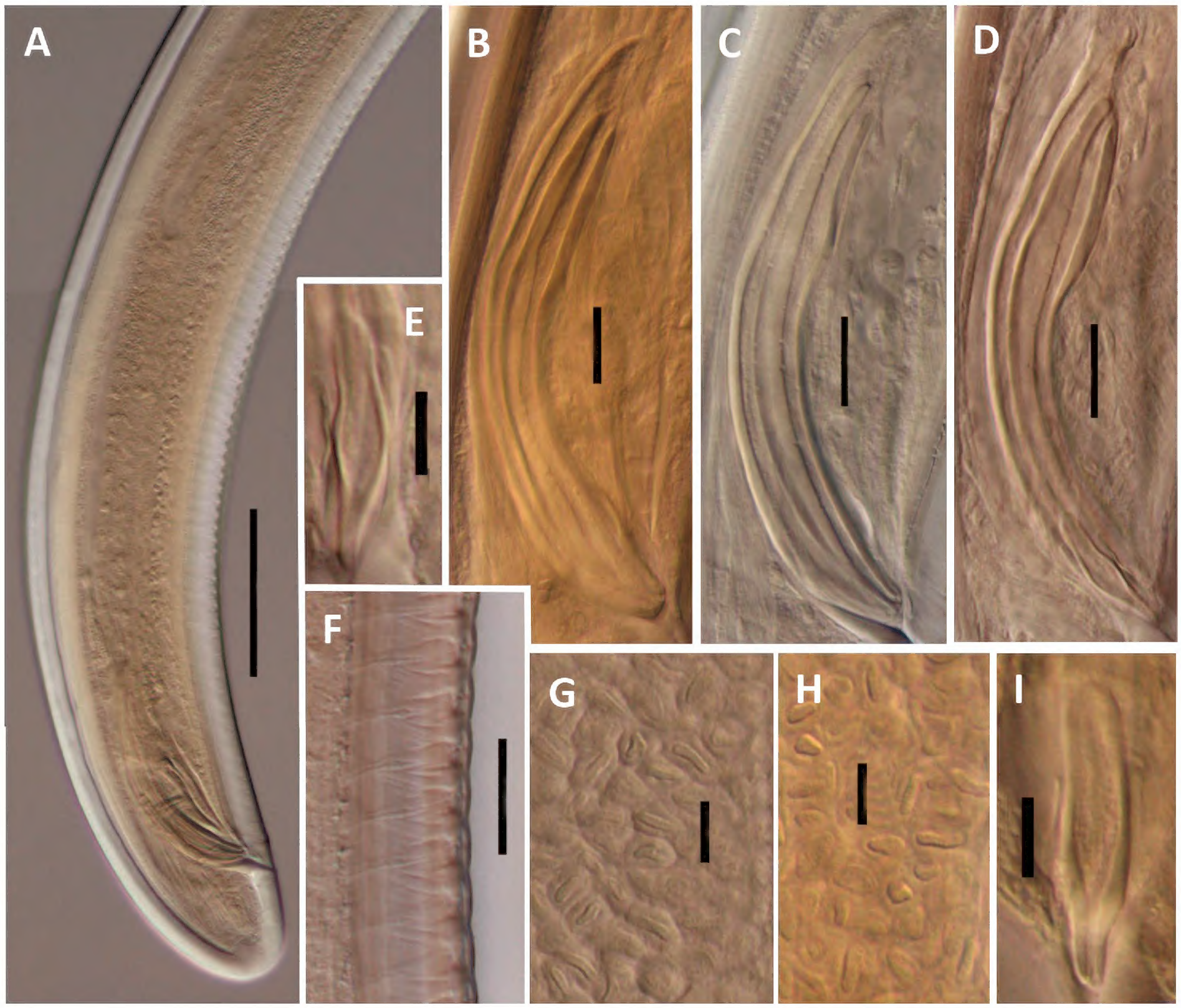

Figure 5. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam (LM, male traits); **A.** Posterior body region; **B–D.** Spicule, arrowheads pointing at the ventral hump; **E, I.** Lateral guiding piece; **F.** Ventromedian supplements. Scale bars: 100 µm (**A**); 20 µm (**B–D, F**); 10 µm (**E, G–I**).

as so usual in dorylaims but appear as short longitudinal slits that resemble the stomata of plant leaves (Fig. 6K–M). Spicules dorylaimid, relatively slender, 6.9–9.1 times longer than wide, 1.8–2.0 times longer than body diameter at level of the cloacal aperture: head 12–25 µm long, occupying up to one-sixth (8–16%) of spicule length, 1.2–1.8 times as long as wide, with its dorsal side visibly longer than the ventral one; median piece 6.5–8.5 µm wide, occupying 32–44% of spicule maximum width; posterior tip 5–9 µm wide; ventral hump and hollow little marked in general, the hump situated at 49–70 µm or 33–47% of spicule length; curvature 118–135°. Lateral guiding pieces 16–24 µm long, 4.0–4.8 times wider than long, almost cylindrical but visibly tapering at its posterior third. Tail similar to that of female, maybe slightly more conoid.

Other populations. (six females and one male from two locations): Morphologically identical to type material. Morphometrically very similar too, with totally coincident or widely overlapping ranges of main measurements and ratios, although a few minor differences are also noted; for instance, prerectum length is appreciably longer in Ha Giang specimens: 324–390 *vs.* 206–321 µm in females and 473 *vs.* 324–390 µm in males.

Molecular characterization. After sequencing and editing, six (three 18S and three 28S) rDNA sequences were obtained of type material for phylogenetic analyses. The three 28S sequences were 798, 679, and 651 bp long, with 511 bp in common, and a Blast search showed that the longest of them was 96.9% similar to that of *Labronema porosum* Vu, Elshishka, Nguyen, Le, Mladenov & Peneva, 2024 (acc. PP060468), 96.3% to that of *L. bidoupense* Vu, Elshishka, Nguyen, Le, Mladenov & Peneva, 2024 (acc. PP060469), 92.0% to that of *Labronema vulvapapillatum* (Meyl, 1954) Loof & Grootaert, 1981 (acc. AY592997), 91.5% to that of *L. vulvapapillatum* (acc. AY592996), 91.3% to that of *Labronema* sp., and 90.8% to that of *Nevadanema nevadense* Álvarez-Ortega & Peña-Santiago, 2012 (acc. JN242245), all of them rounded-tailed representatives of Dorylaimidae de Man, 1876, Labronematinae Peña-Santiago & Álvarez-Ortega, 2014.

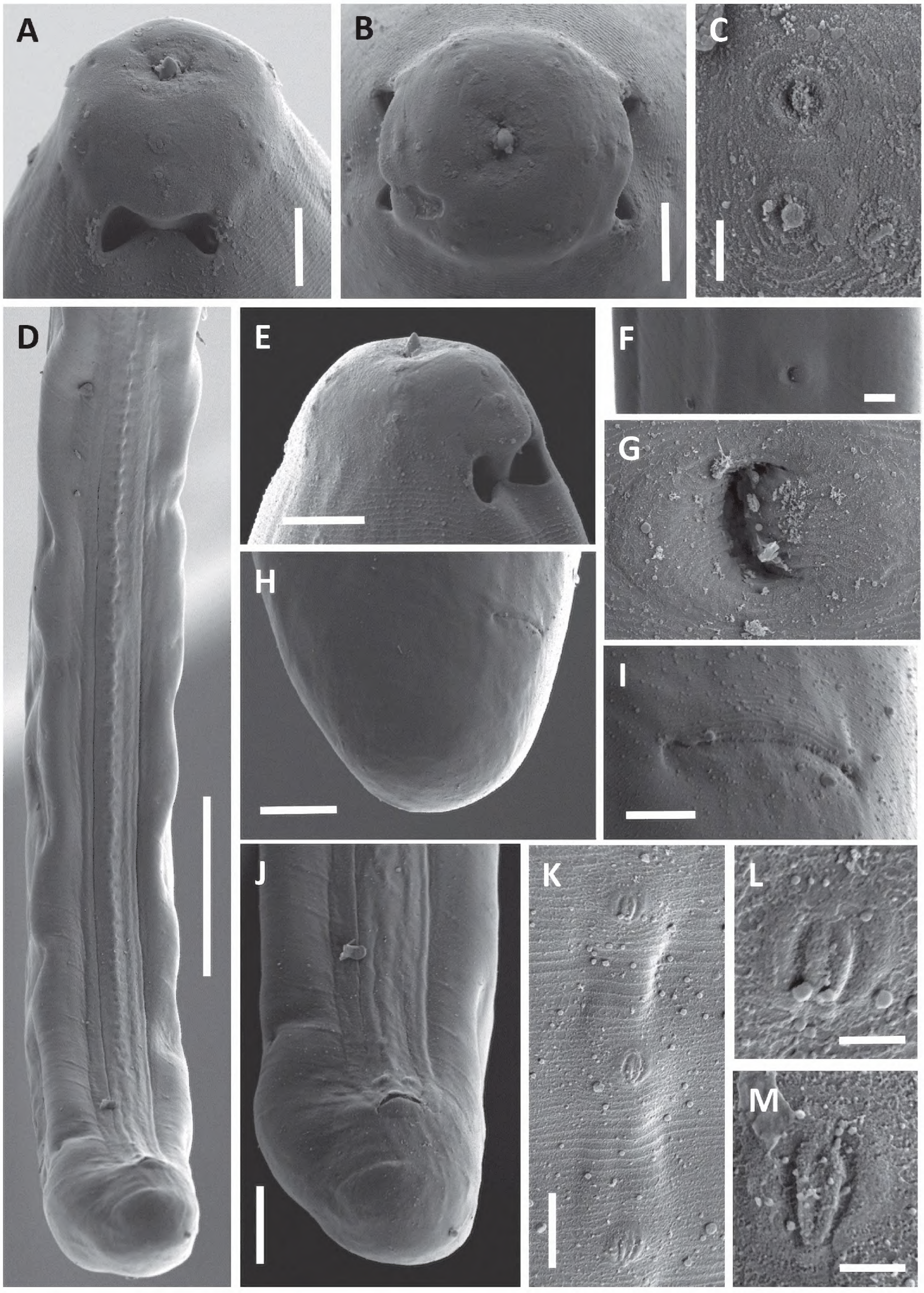

Figure 6. *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam (SEM); **A.** Lip region, lateral view; **B.** Lip region, in face view; **C.** Labial papillae; **D.** Male, posterior body region, ventral view; **E.** Lip region, subventral view; **F, G.** Vulva, ventral view; **H.** Female, subventral view; **I.** Anus; **J.** Male, caudal region; **K–M.** Detail of ventromedian supplements. Scale bars: 5 µm (**A, B, E, I**); 1 µm (**C**); 100 µm (**D**); 10 µm (**F**); 4 µm (**G**); 20 µm (**H, J**); 3 µm (**K**); 1 µm (**L, M**).

Table 1. Main morphometrics of *Vastnema crassicutaneum* gen. et sp. nov. from Vietnam. Measurements in µm except L in mm, in the form: average ± sd (range).

Character	Population	Nam Xuan Lac, Bac Can			Du Gia, Ha Giang		Cuc Phuong, Ninh Binh
		Holotype	Paratypes	Paratypes			
	n	♀	11♀♀	7♂♂	4♀♀	♂	2♀♀
L		6.19	5.51 ± 0.47 (5.00–6.35)	5.18 ± 0.26 (4.79–5.48)	5.80 ± 0.37 (5.26–6.09)	5.66	5.25, 5.63
a		31	29.4 ± 2.7 (25–34)	31.1 ± 2.4 (27–34)	32.2 ± 1.8 (30–34)	33	29, 40
b		7.7	7.1 ± 0.7 (6.3–8.4)	7.2 ± 0.8 (6.3–8.7)	7.3 ± 0.1 (7.2–7.5)	7.6	7.0, 6.3
c		129	113 ± 11 (98–131)	107 ± 17 (94–140)	116 ± 22 (101–142)	107	103, 106
V		41	41.8 ± 1.6 (39–44)	-	39.0 ± 1.2 (38–41)	-	?, 44
c'		0.5	0.5 ± 0.0 (0.5–0.6)	0.6 ± 0.0 (0.5–0.7)	0.5 ± 0.1 (0.5–0.6)	0.7	0.5, 0.5
Lip region diameter		31	29.3 ± 1.4 (27–31)	28.1 ± 1.3 (26–30)	27.0 ± 1.2 (26–28)	28	31, 31
Odontostyle length		55	55.2 ± 2.7 (51–60)	53.4 ± 2.6 (49–57)	55.3 ± 2.8 (52–58)	55	51, 70
Odontophore length		80	77.7 ± 4.0 (72–84)	75.9 ± 3.8 (70–79)	73–0 ± 2.4 (70–76)	77	73, 82
Neck length		803	777 ± 54 (627–821)	720 ± 44 (631–755)	794 ± 59 (706–837)	746	746, 888
Pharyngeal expansion length		386	370 ± 29 (292–390)	341 ± 22 (299–364)	386 ± 42 (338–418)	357	357, 449
Body diameter at neck base		167	148 ± 10 (133–167)	136 ± 12 (122–156)	148 ± 4.9 (145–154)	138	142, 137
mid-body		200	188 ± 11 (172–210)	168 ± 20 (144–194)	180 ± 4.3 (175–185)	170	179, 141
anus		96	94.1 ± 4.0 (88–102)	79.0 ± 3.9 (71–84)	94.3 ± 1.5 (93–96)	76	99, 99
Distance vulva – anterior end		2564	2300 ± 193 (2035–2585)	-	2256 ± 91 (2143–2344)	-	?, 2491
Prerectum length		321	259 ± 39 (206–321)	339 ± 57 (265–412)	361 ± 30 (324–390)	473	319, 228
Rectum/cloaca length		121	89.9 ± 11.8 (79–121)	108 ± 7 (96–117)	74 ± 5.6 (68–79)	98	97, 90
Tail length		48	48.8 ± 3.1 (45–56)	49.0 ± 5.1 (39–54)	50.3 ± 6.7 (43–56)	53	51, 53
Spicules length		-	-	149 ± 7 (139–160)	-	138	-
Ventromedian supplements		-	-	47–58	-	45	-

The three 18S sequences were 1698, 1690, and 1689 bp long, with 1684 bp in common, and a Blast search showed that the longest of them was 97.2% similar to that of *Aporcelaimellus* sp. (acc. JX674034), 97.2% to that of *L. ferox* Thorne, 1939 (acc. AY552972), 97.2% to that of *Aporcelaimellus* sp. (acc. PP099686), 97.1% to that of *Prodorylaimus* sp. (EF207246), and 97.0% to that of *Mesodorylaimus* sp. (acc. MG921256).

Diagnosis. The new species is characterized by its 4.79–6.35 mm long body, cuticle three-layered and 12.5–15.5 µm thick at anterior region, lip region continuous with the adjoining body and 26–31 µm wide, odontostyle 49–60 µm long or 1.8–2.0 times the lip region diameter, neck 627–888 µm long, pharyngeal expansion occupying 46–49% of the total neck length, female genital system diovarian, uterus complex and 571–804 µm or 3.2–4.3 body diameters long, pre-equatorial (V = 38–44) and longitudinal vulva, tail short and rounded in both sexes (39–56 µm, c = 98–140, c' = 0.5–0.7), spicules 138–160 µm long, and 45–58 small ventromedian supplements with hiatus.

Separation from some resembling species. The new species resembles a few *Labronema* species. In having large size, it compares to *L. magnum* Altherr, 1972, a freshwater species known to occur in Nepal, Russia, and Sweden (see taxonomic revision by Peña-Santiago, 2022), but it differs from this in its much thicker cuticle (*vs.* for instance, 4–5 µm at anterior region), lip region with different shape (*vs.* anterior region truncate, offset by weak constriction, and one-third as wide as body diameter at neck base), comparatively much shorter neck (*vs.* b = 4.1–6.3), more anterior vulva (*vs.* V = 44–53), longer spicules (*vs.* 118–135 µm), and more numerous (*vs.* 21–31) ventromedian supplements with different aspect (*vs.* typically mammiform).

In having continuous lip region, it also resembles several atypical *Labronema* representatives, including *L. hyalinum* (Thorne & Swanger, 1936), Thorne, 1939, *L. neopacificum* Rahman, Jairajpuri, Ahmad & Ahmad, 1987, *L. orientale* Andrássy, 2011, *L. singhalense* Andrássy, 2011, *L. stechlinense* Altherr, 1968, and *L. thornei* Ferris, 1968, but all of them show smaller general size (body length up to 3.73 mm long, odontostyle up to 48 µm long), comparatively longer neck (b-ratio up to 5.2), more posterior vulva (V = 45–55), shorter spicules (up to 120 µm long), and much less ventromedian supplements (up to 29) with different shapes.

Type material. Female holotype, seven female and three male paratypes, and one male paratype deposited at the Department of Nematology, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam. Four (two female and two male) paratypes at the Nematode Collection of the University of Jaén, Spain. Four (two female and two male) paratypes at USDANC, Beltsville, Maryland, USA.

Etymology. The species epithet derives from the Latin terms *crassus* = thick and *cutaneum* = skin, cuticle, and refers to this peculiar trait of the new taxon.

Evolutionary relationships of the new taxon

Morphologically, *Vastnema crassicutaneum* gen. et sp. nov. is characterized by a unique combination of very unusual traits within the family Dorylaimidae de Man, 1876, and even the order Dorylaimida. First, its very thick, three-layered cuticle is only observed in some very large dorylaims and should be regarded, with a cladistic approach, as a relevant apomorphy. Second, its comparatively short pharynx/neck, with b-ratio always exceeding 6.0 and very often appreciably more, probably is an

autapomorphy of the new taxon, especially if its rounded-tailed condition is considered. Third, the presence of an exclusive uterine specialization, resembling those known as Z-like structures but significantly different from them in its nature, is another recognizable autapomorphic feature too. Fourth, the high number and, in particular, the shape of male ventromedian supplements, appearing as plant leaf stomata rather than button-like or mammiform structures, certainly is (at least) an infrequent apomorphy. Other interesting traits (lip region continuous and with totally fused lips, double guiding ring, pre-equatorial vulva, rounded-tailed sexes) are more usual apomorphic features within dorylaims. As mentioned, the new taxon shares several traits (strong odontostyle, double guiding ring, rounded tail in both sexes) with members of the subfamily Labronematinae, in particular *Nevadanema* and some *Labronema* representatives, supporting a close relationship between them.

As derived from the molecular analyses of obtained sequences, whose results are presented in Figs 7 (18S tree), 8 (28S tree), the evolutionary relationships of the new taxon basically agree with those deduced from morphological observations. Thus, and in both trees, *Vastnema* gen. nov. sequences form part of a maximally (100%) supported clade also including sequences of *Labronema* representatives. Moreover, the 28S tree, which provides better general resolution, shows that the (*Vastnema* gen. nov. + *Labronema* spp.) clade belongs to a much larger (highlighted in yellow, and also maximally supported) clade containing sequences of Dorylaimidae and Actinolaimidae Thorne, 1939 taxa. It is remarkable that this large subclade is divided into two subclades,

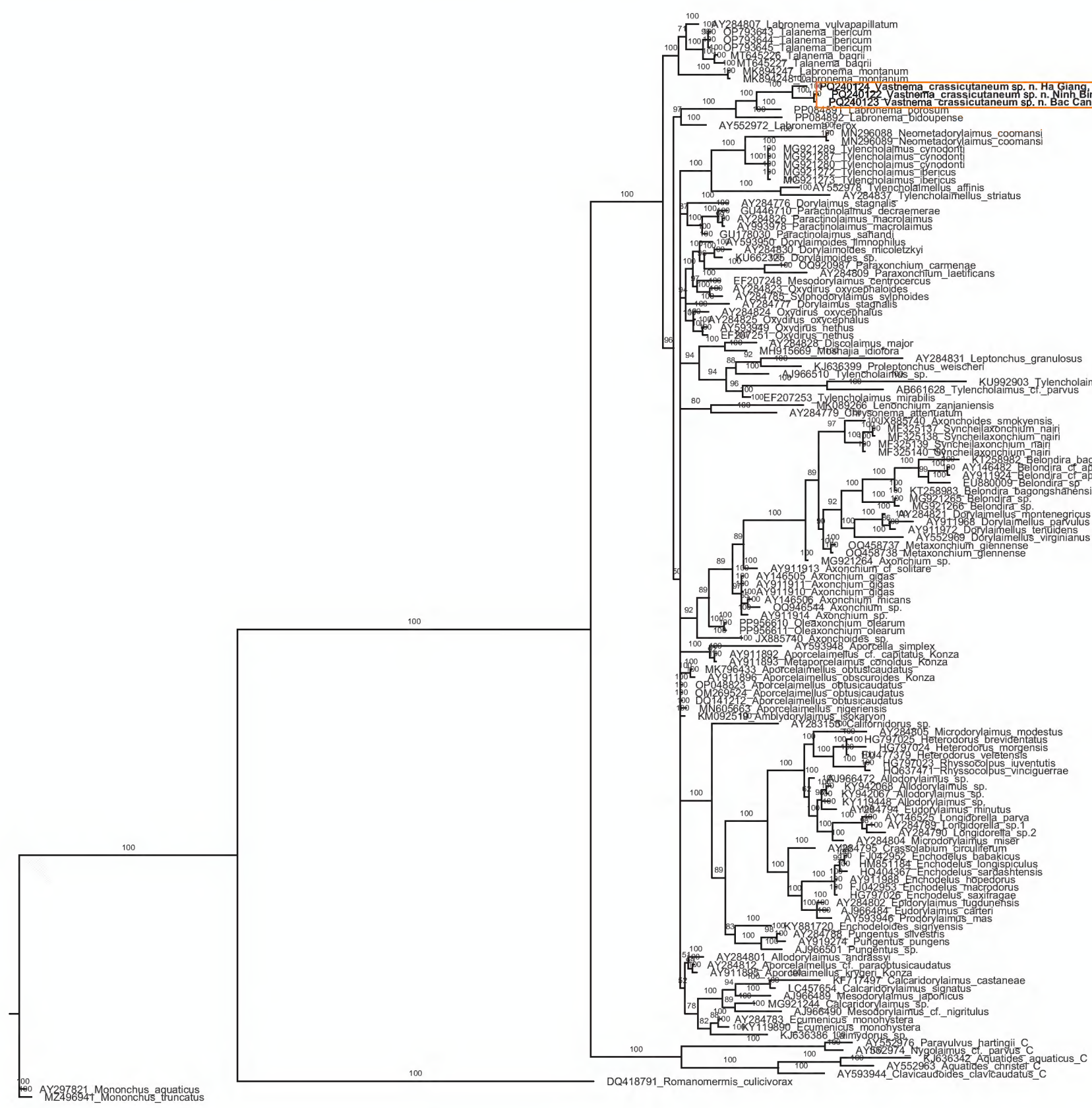

Figure 7. Bayesian inference tree from the newly sequenced *Vastnema crassicutaneum* gen. et sp. nov. based on sequences of the 18S rDNA region. Bayesian posterior probabilities (%) are given for each clade. The scale bar shows the number of substitutions per site.

Figure 8. Bayesian inference tree from the newly sequenced *Vastnema crassicutaneum* gen. et sp. nov. based on sequences of the 28S rDNA region. Bayesian posterior probabilities (%) are given for each clade. The scale bar shows the number of substitutions per site.

one including sequences of *Vastnema* gen. nov., *Labronema*, *Nevadanema*, and *Prodorylaimus* sp., and another consisting of sequences of *Dorylaimus stagnalis* Dujardin, 1845, plus several actinolaims.

An integrative approach to the subject, combining morphological and molecular information, supports a very close relationship of the new taxon with Labronematinae representatives, but also that internal and external relationships of Dorylaimidae might be more complex than traditionally assumed, a question that will require much further research before its elucidation.

Acknowledgements

The senior author (RPS) deeply appreciates the invitation, support, and reception of the Department of Nematology, Institute of Ecology and Biological Resources

(IEBR), Vietnamese Academy of Sciences and Technology (VAST), Hanoi, Vietnam, to visit its lab and to participate in the project NCXS01.04/23-25. The Spanish authors (JA, RPS) thank the staff (Amparo Martínez-Morales), and this study used the facilities of the equipment of "Centro de Instrumentación Científico-Técnica (CICT)," University of Jaén, for assistance to obtain SEM pictures.

The work is supported by the Vietnam Academy of Science and Technology (VAST) under the project NCXS01.04/23-25 "Developing the first-class research team on the discovery of diversity and application potential of hymenopterans, myriapods, and soil nematodes in the limestone mountains of northeastern Vietnam."

References

Altherr E (1968) Nématodes de la nappe phréatique du réseau fluvial de la Saale (Thuringe) et psammniques du Lac Stechlin (Brandebourg dur nord). Limnologica 6: 247–320.

Altherr E (1972) Contribution à la connaissance des Nématodes rithrostygopsammiques et rithrostygopséphiques de Suède. Revue Suisse de Zoologie 79: 881–902. https://doi.org/10.5962/bhl.part.97141

Álvarez-Ortega S, Peña-Santiago R (2012) Nematodes of the order Dorylaimida from Andalucía Oriental, Spain. *Nevadanema nevadense* gen. n., sp. n. (Qudsianematidae) from Sierra Nevada National Park. Nematology 14: 249–264. https://doi.org/10.1163/138855411X589309

Álvarez-Ortega S, Nguyen, TAD, Abolafia J, Vu TTT, Peña-Santiago R (2015) Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus. Zookeys 516: 1–26. https://doi.org/10.3897/zookeys.516.10087

Álvarez-Ortega S, Nguyen TAD, Abolafia J, Vu TTT, Bonkowski M, Peña-Santiago R (2016) Three new species of *Sectonema* Thorne, 1930 (Dorylaimida, Aporcelaimidae) from Vietnam. Nematology 18: 517–536. https://doi.org/10.1163/15685411-00002974

Andrássy I (2009) Free-living nematodes of Hungary. III. Pedozoologica Hungarica n° 5. Hungarian Natural History Museum. Budapest, Hungary, 608 pp.

Andrássy I (2011) Three new bisexual species of *Labronema* Thorne, 1939 (Nematoda, Qudsianematidae). Opuscula Zoologica Budapestinensis 43: 107–120. https://www.oalib.com/research/2072275

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Dujardin F (1845) Histoire naturelle des helminthes ou vers intestinaux. Librairie Encyclopédique de Roret, Paris, 654 pp.

Ferris VR (1968) Biometric analyses in the genus *Labronema* (Nematoda, Dorylaimida) with a description of *L. thornei* n. sp. Nematologica 14: 276–284. https://doi.org/10.1163/187529268X00480

Floyd RM, Rogers AD, Lambshead PJD, Smith CR (2005) Nematode-specific PCR primers for the 18S small subunit rRNA gene. Molecular Ecology Notes 5(3): 611–612. https://doi.org/10.1111/j.1471-8286.2005.01009.x

Hoang H, Chu HH, Nguyen TAD, Trinh QP, Abolafia J, Peña-Santiago R (2019) Morphological and molecular characterisation of *Chitwoodius coffeae* sp. n. (Dorylaimida, Tylencholaimidae) from Vietnam, with a revised taxonomy of the genus. Nematology 21: 509–521. https://doi.org/10.1163/15685411-00003231

Holterman M, Wurff A, Elsen S, Megen H, Bongers T, Holovachov O, Bakker J, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Molecular Biology and Evolution 23: 1792–1800. https://doi.org/10.1093/molbev/msl044

Kumar S, Stecher G, Tamura K (2021) MEGA 11: Molecular Evolutionary Genetics Analysis version 11.0. Molecular Biology and Evolution 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Larget B, Simon DL (1999) Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. Molecular Biology and Evolution 16: 750–759. https://doi.org/10.1093/oxfordjournals.molbev.a026160

Loof PAA, Grootaert P (1981) Redescription of *Labronema vulvapapillatum* Meyl, (1954) nov. comb. (Dorylaimoidea). Nematologica 27: 139–145. https://doi.org/10.1163/187529281X00197

Man J G de (1876) Onderzoekingen over vrij in de aarde levende Nematoden. Tijdschrift Nederlandsche dierkundige Vereeniging 2: 78–196.

Meyl AH (1954) Die bisher in Italien gefundenen freilebenden Erd und Süsswasser-Nematoden. Archivio Zoologico Italiano, Torino 39: 161–264.

Nguyen TAD, Peña-Santiago R (2018) Two new species of the genus *Metaxonchium* Coomans & Nair, 1975 (Nematoda, Dorylaimida, Belondiridae) from Vietnam. Zootaxa 4415: 150–160. https://doi.org/10.11646/zootaxa.4415.1.7

Nguyen TAD, Peña-Santiago R (2020) Morphological and molecular characterization of *Epidorylaimus procerus* sp. n. (Dorylaimida, Qudsianematidae) from Vietnam. Journal of Nematology 52: e2020–112 [8 pp]. https://doi.org/10.21307/jofnem-2020-112

Nguyen TAD, Vu TTT, Bonkowski M, Peña-Santiago R (2014) New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979. Biodiversity Data Journal 2: e1156. https://doi.org/10.3897/BDJ.2.e1156

Nguyen TAD, Abolafia J, Bonkowski M, Peña-Santiago R (2016) Two new species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. Zootaxa 4103: 550–560. https://doi.org/10.11646/zootaxa.4103.6.5

Nguyen TAD, Abolafia J, Dumack K, Bonkowski M, Peña-Santiago R (2017) Two known species of *Aporcelinus* Andrássy, 2009 (Dorylaimida, Aporcelaimidae) from Vietnam, with the first molecular study of the genus. Nematology 19: 853–868. https://doi.org/10.1163/15685411-00003092

Peña-Santiago R (2021) Morphology and bionomics of dorylaims (Nematoda: Dorylaimida). Nematology Monographs and Perspectives 13 (Series Editors: Cook, R. & Hunt, D.J.). Brill. Leiden, The Netherlands, 278 pp. https://doi.org/10.1163/9789004447851

Peña-Santiago R (2022) New synonyms in the genus *Labronema* Thorne, 1939 (Nematoda, Dorylaimida, Dorylaimidae). Nematology 24: 953–957. https://doi.org/10.1163/15685411-bja10175

Peña-Santiago R, Álvarez-Ortega S (2014) An integrative approach to assess the phylogeny and the systematics of rounded-tailed genera of the subfamily Qudsianematinae (Nematoda, Dorylaimida). Zoologica Scripta 43: 418–428. https://doi.org/10.1111/zsc.12058

Rahman MF, Jairajpuri MS, Ahmad I, Ahmad W (1987) Two new species of *Labronema* Thorne, 1939 (Nematoda, Dorylaimida) from India. Nematologica 32(1986): 367–373. https://doi.org/10.1163/187529286X00264

Rambaut A (2018) Figtree, a graphical viewer of phylogenetic trees. https://github.com/rambaut/figtree/releases/tag/v1.4.4

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica 4: 67–69. https://doi.org/10.1163/187529259X00381

Seinhorst JW (1962) On the killing, fixation and transferring to glycerine of nematodes. Nematologica 8: 29–32. https://doi.org/10.1163/187529262X00981

Southey JF (1986) Laboratory methods for work with plant and soil nematodes. London: Her Majesty' Stationery Office, 202 pp.

Subbotin SA, Sturhan D, Chizhov VN, Vovlas N, Baldwin JG (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rDNA gen sequences. Nematology 8: 455–74. https://doi.org/10.1163/156854106778493420

Thorne G (1939) A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8: 1–261.

Thorne G, Swanger HH (1936) A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n. gen., *Dorylaimoides* n. gen., and *Pungentus* n. gen. Capita Zoologica 6: 1–223.

Vu TTT, Elshishka M, Nguyen AD, Le TML, Mladenov A, Peneva V (2024) Two new species of genus *Labronema* Thorne, 1939 (Nematoda, Dorylaimidae) from natural parks of Vietnam with an identification key to the species with a medium-sized odontostyle. Zookeys 1215: 1–26. https://doi.org/10.3897/zookeys.1215.128183

Yoder M, De Ley I T, King I, Mundo-Ocampo M, Mann J, Blaxter M, Poiras L, De Ley P (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology 8: 367–376. https://doi.org/10.1163/156854106778493448